

WHAT IS CLAIMED IS:

- 1           1.       An isolated infectious recombinant respiratory syncytial virus (RSV)  
2       comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a  
3       nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase  
4       elongation factor, wherein a modification is introduced in the genome or antigenome  
5       comprising a partial or complete deletion of M2 ORF2 or one or more nucleotide  
6       change(s) that reduce or ablate expression of M2 ORF2.
- 1           2.       The recombinant RSV of claim 1, wherein expression of M2 ORF2 is  
2       ablated by introduction of one or more stop codons.
- 1           3.       The recombinant RSV of claim 2 which is rA2-K5.
- 1           4.       The recombinant RSV of claim 1, wherein expression of M2 ORF2 is  
2       ablated by introduction of a frame shift mutation.
- 1           5.       The recombinant RSV of claim 4 which is rA2-NdeI.
- 1           6.       The recombinant RSV of claim 1, wherein M2 ORF2 is deleted in whole  
2       or in part.
- 1           7.       The recombinant RSV of claim 1, wherein the modification in the genome  
2       or antigenome specifies one or more desired phenotypic changes in the recombinant RSV  
3       selected from (i) a change in mRNA synthesis, (ii) a change in the level of viral protein  
4       expression; (iii) a change in genomic or antigenomic RNA replication, (iv) a change in  
5       viral growth characteristics, (v), a change in viral plaque size, and/or (vi) a change in  
6       cytopathogenicity.
- 1           8.       The recombinant RSV of claim 7, wherein the phenotypic change  
2       comprises attenuation of viral growth compared to growth of a corresponding wild-type  
3       or mutant parental RSV strain.
- 1           9.       The recombinant RSV of claim 1, wherein the RSV genome comprises one  
2       or more shifted RSV gene(s) or genome segment(s) that is/are positionally shifted within  
3       genome or antigenome to a more promoter-proximal or promoter-distal position relative

4 to a position of said RSV gene(s) or genome segment(s) within a wild type RSV genome  
5 or antigenome.

1 10. The recombinant RSV of claim 9, wherein said one or more shifted  
2 gene(s) or genome segment(s) is/are shifted to a more promoter-proximal or promoter-  
3 distal position by deletion or insertion of one or more displacement polynucleotide(s)  
4 within said partial or complete genome or antigenome.

1 11. The recombinant RSV of claim 7, wherein the phenotypic change  
2 comprises delayed kinetics of viral mRNA synthesis compared to kinetics of mRNA  
3 synthesis of a corresponding wild-type or mutant parental RSV strain.

1 12. The recombinant RSV of claim 7, wherein the phenotypic change  
2 comprises a change in cumulative mRNA synthesis compared to cumulative mRNA  
3 synthesis of a corresponding wild-type or mutant parental RSV strain.

1 13. The recombinant RSV of claim 12, wherein the increase in cumulative  
2 viral mRNA synthesis is approximately 1.3 to 2-fold or greater at 24 hours post-infection  
3 compared to cumulative mRNA synthesis of the corresponding wild-type or mutant  
4 parental RSV strain.

1 14. The recombinant RSV of claim 7, wherein the phenotypic change  
2 comprises increased viral protein accumulation in infected cells compared to viral protein  
3 accumulation in cells infected with a corresponding wild-type or mutant parental RSV  
4 strain.

1 15. The recombinant RSV of claim 7, wherein accumulation of one or more  
2 viral proteins is increased approximately 2- to 3-fold or greater compared to viral protein  
3 accumulation in cells infected with the corresponding wild-type or mutant parental RSV  
4 strain.

1 16. The recombinant RSV of claim 7, wherein the phenotypic change  
2 comprises increased expression of one or more viral antigens compared to expression of  
3 said one or more viral antigens by the corresponding wild-type or mutant parental RSV  
4 strain.

- 1           17.     The recombinant RSV of claim 7, wherein the phenotypic change  
2 comprises a change in viral RNA replication compared to viral RNA replication of a  
3 corresponding wild-type or mutant parental RSV strain.
- 1           18.     The recombinant RSV of claim 17, wherein accumulation of genomic and  
2 antigenomic RNA is decreased compared to accumulation of genomic and antigenomic  
3 RNA of the corresponding wild-type or mutant parental RSV strain.
- 1           19.     The recombinant RSV of claim 7, wherein the phenotypic change  
2 comprises an increase in cumulative mRNA synthesis and a reduction in viral RNA  
3 replication compared to cumulative mRNA synthesis and viral RNA replication of a  
4 corresponding wild-type or mutant parental RSV strain.
- 1           20.     The recombinant RSV of claim 19, wherein a cumulative molar ratio of  
2 mRNA to genomic RNA is increased approximately 7- to 18-fold or greater compared to  
3 a cumulative molar ratio of mRNA to genomic RNA observed for the corresponding  
4 wild-type or mutant parental RSV strain.
- 1           21.     The recombinant RSV of claim 7, wherein the phenotypic change  
2 comprises a larger plaque phenotype compared to plaque phenotype of a corresponding  
3 wild-type or mutant parental RSV strain.
- 1           22.     The recombinant RSV of claim 7, wherein the phenotypic change  
2 comprises a change in cytopathogenicity compared to cytopathogenicity of a  
3 corresponding wild-type or mutant parental RSV strain.
- 1           23.     The recombinant RSV of claim 1, wherein the genome or antigenome is  
2 further modified by introduction of one or more attenuating mutations identified in a  
3 biologically derived mutant human RSV.
- 1           24.     The recombinant RSV of claim 23, wherein the genome or antigenome  
2 incorporates at least one and up to a full complement of attenuating mutations present  
3 within a panel of biologically derived mutant human RSV strains, said panel comprising  
4 cpts RSV 248 (ATCC VR 2450), cpts RSV 248/404 (ATCC VR 2454), cpts RSV  
5 248/955 (ATCC VR 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV 530/1009 (ATCC

6 VR 2451), cpts RSV 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5 (ATCC VR 2542),  
7 and RSV B-1 cp-23 (ATCC VR 2579).

1 25. The recombinant RSV of claim 23, wherein the genome or antigenome  
2 incorporates at least one and up to a full complement of attenuating mutations specifying  
3 an amino acid substitution at Val267 in the RSV N gene, Glu218 and/or Thr523 in the  
4 RSV F gene, Asn43, Cys319 Phe 521, Gln831, Met1169, Tyr1321 and/or His 1690 in the  
5 RSV polymerase gene L, and a nucleotide substitution in the gene-start sequence of gene  
6 M2.

1 26. The recombinant RSV of claim 23, wherein the genome or antigenome  
2 incorporates at least two attenuating mutations.

1 27. The recombinant RSV of claim 23, wherein the genome or antigenome  
2 includes at least one attenuating mutation stabilized by multiple nucleotide changes in a  
3 codon specifying the mutation.

1 28. The recombinant RSV of claim 1, wherein the genome or antigenome  
2 comprises an additional nucleotide modification specifying a phenotypic change selected  
3 from a change in growth characteristics, attenuation, temperature-sensitivity, cold-  
4 adaptation, plaque size, host-range restriction, antigen expression, or a change in  
5 immunogenicity.

1 29. The recombinant RSV of claim 28, wherein the additional nucleotide  
2 modification alters a SH, NS1, NS2, or G gene of the recombinant RSV.

1 30. The recombinant RSV of claim 29, wherein a SH, NS1, NS2, or G gene is  
2 deleted in whole or in part or expression of the gene is reduced or ablated by a frame shift  
3 or introduction of one or more stop codons in an open reading frame of the gene or a  
4 modification of a translational start site.

1 31. The recombinant RSV of claim 28, wherein the nucleotide modification  
2 comprises a nucleotide deletion, insertion, substitution, addition or rearrangement of a  
3 cis-acting regulatory sequence of a selected gene within the recombinant RSV genome or  
4 antigenome.

- 1           32.     The recombinant RSV of claim 31, wherein a gene end (GE) signal of the  
2     NS1 or NS2 gene is modified.
- 1           33.     The recombinant RSV of claim 28, wherein the nucleotide modification  
2     comprises an insertion, deletion, substitution, or rearrangement of a translational start site  
3     within the recombinant RSV genome or antigenome.
- 1           34.     The recombinant RSV of claim 33, wherein the translational start site for a  
2     secreted form of the RSV G glycoprotein is ablated.
- 1           35.     The recombinant RSV of claim 28, wherein the genome or antigenome is  
2     modified to encode a non-RSV molecule selected from a cytokine, a T-helper epitope, a  
3     restriction site marker, or a protein of a microbial pathogen capable of eliciting a  
4     protective immune response in a mammalian host.
- 1           36.     The recombinant RSV of claim 28, wherein the genome or antigenome  
2     incorporates a gene or genome segment from parainfluenza virus (PIV).
- 1           37.     The recombinant RSV of claim 36, wherein the gene or genome segment  
2     encodes a PIV HN or F glycoprotein or immunogenic domain or epitope thereof.
- 1           38.     The recombinant RSV of claim 37, wherein the genome segment encodes  
2     an ectodomain or immunogenic epitope of HN or F of PIV1, PIV2, or PIV3.
- 1           39.     The recombinant RSV of claim 1, wherein the genome or antigenome  
2     comprises a partial or complete RSV background genome or antigenome of a human or  
3     bovine RSV combined with a heterologous gene or genome segment of a different RSV  
4     to form a human-bovine chimeric RSV genome or antigenome.
- 1           40.     The recombinant RSV of claim 39, wherein the heterologous gene or  
2     genome segment encodes a RSV F, G or SH glycoprotein or an immunogenic domain or  
3     epitope thereof.
- 1           41.     The recombinant RSV of claim 39, wherein the heterologous gene or  
2     genome segment is substituted for a counterpart gene or genome segment in a partial RSV  
3     background genome or antigenome

- 1           42.     The recombinant RSV of claim 39, wherein the heterologous gene or  
2 genome segment is added adjacent to or within a noncoding region of the partial or  
3 complete RSV background genome or antigenome
- 1           43.     The recombinant RSV of claim 39, wherein the chimeric genome or  
2 antigenome comprises a partial or complete human RSV background genome or  
3 antigenome combined with a heterologous gene or genome segment from a bovine RSV
- 1           44.     The recombinant RSV of claim 39, wherein the chimeric genome or  
2 antigenome comprises a partial or complete bovine RSV background genome or  
3 antigenome combined with a heterologous gene or genome segment from a human RSV
- 1           45.     The recombinant RSV of claim 44, wherein one or more human RSV  
2 glycoprotein genes F, G and SH or a genome segment encoding a cytoplasmic domain,  
3 transmembrane domain, ectodomain or immunogenic epitope thereof is substituted for a  
4 counterpart gene or genome segment within the bovine RSV background genome or  
5 antigenome
- 1           46.     The recombinant RSV of claim 45, wherein one or both human RSV  
2 glycoprotein genes F and G is substituted to replace one or both counterpart F and G  
3 glycoprotein genes in the bovine RSV background genome or antigenome.
- 1           47.     The recombinant RSV of claim 46, wherein both human RSV glycoprotein  
2 genes F and G are substituted to replace counterpart F and G glycoprotein genes in the  
3 bovine RSV background genome or antigenome.
- 1           48.     The recombinant RSV of claim 45, wherein the heterologous gene or  
2 genome segment is from a subgroup A or subgroup B human RSV.
- 1           49.     The recombinant RSV of claim 45, wherein the human-bovine chimeric  
2 genome or antigenome incorporates antigenic determinants from both subgroup A and  
3 subgroup B human RSV.
- 1           50.     The recombinant RSV of claim 1 which is a virus.
- 1           51.     The recombinant RSV of claim 1 which is a subviral particle.

- 1            52.    A method for stimulating the immune system of an individual to induce  
2 protection against RSV which comprises administering to the individual an  
3 immunologically sufficient amount of the recombinant RSV of claim 1 combined with a  
4 physiologically acceptable carrier.
- 1            53.    The method of claim 52, wherein the recombinant RSV is administered in  
2 a dose of  $10^3$  to  $10^7$  PFU.
- 1            54.    The method of claim 52, wherein the recombinant RSV is administered to  
2 the upper respiratory tract.
- 1            55.    The method of claim 52, wherein the recombinant RSV is administered by  
2 spray, droplet or aerosol.
- 1            56.    The method of claim 52, wherein the recombinant RSV is administered to  
2 an individual seronegative for antibodies to RSV or possessing transplacentally acquired  
3 maternal antibodies to RSV.
- 1            57.    The method of claim 52, wherein the recombinant RSV is attenuated and  
2 exhibits increased antigen expression compared to growth and antigen expression of a  
3 corresponding wild-type or mutant parental RSV strain.
- 1            58.    The method of claim 47, wherein the recombinant RSV elicits an immune  
2 response against human RSV A, human RSV B, or both.
- 1            59.    An immunogenic composition to elicit an immune response against RSV  
2 comprising an immunologically sufficient amount of the recombinant RSV of claim 1 in a  
3 physiologically acceptable carrier.
- 1            60.    The immunogenic composition of claim 62, formulated in a dose of  $10^3$  to  
2  $10^7$  PFU.
- 1            61.    The immunogenic composition of claim 59, formulated for administration  
2 to the upper respiratory tract by spray, droplet or aerosol.

1           62.     The immunogenic composition of claim 59, wherein the recombinant RSV  
2 exhibits attenuated growth and increased antigen expression compared to growth and  
3 antigen expression of a corresponding wild-type or mutant parental RSV strain.

1           63.     The immunogenic composition of claim 62 which elicits an immune  
2 response against human RSV A, human RSV B, or both.

1           64.     An isolated polynucleotide molecule comprising a RSV genome or  
2 antigenome which is modified by a partial or complete deletion of M2 ORF2 or one or  
3 more nucleotide changes that reduce or ablate expression of M2 ORF2.

1           65.     The isolated polynucleotide molecule of claim 64, wherein one or more  
2 stop codons are introduced in M2 ORF2.

1           66.     The isolated polynucleotide molecule of claim 64, wherein a frame shift  
2 mutation is introduced in M2 ORF2.

1           67.     The isolated polynucleotide molecule of claim 64 which incorporates NdeI  
2 or K5 mutations.

1           68.     The isolated polynucleotide molecule of claim 64, wherein the genome or  
2 antigenome is further modified by introduction of one or more attenuating mutations  
3 identified in a biologically derived mutant human RSV wherein both human RSV  
4 glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein  
5 genes in the bovine RSV genome or antigenome.

1           69.     The isolated polynucleotide molecule of claim 64, wherein the genome or  
2 antigenome comprises an additional nucleotide modification specifying a phenotypic  
3 change selected from a change in growth characteristics, attenuation, temperature-  
4 sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in  
5 immunogenicity.

1           70.     The isolated polynucleotide molecule of claim 69, wherein the genome or  
2 antigenome is modified by deletion of a SH, NS1, NS2, G gene in whole or in part or by  
3 introduction of a frame shift or stop codon in an open reading frame of the gene that  
4 reduces or ablates gene expression.



- 1           71.     The isolated polynucleotide molecule of claim 70, wherein a SH, NS1,  
2     NS2, or G gene is deleted in whole or in part.
- 1           72.     The isolated polynucleotide molecule of claim 69, wherein the nucleotide  
2     modification comprises a nucleotide deletion, insertion, addition or rearrangement of a  
3     cis-acting regulatory sequence of a selected RSV gene within the RSV genome or  
4     antigenome.
- 1           73.     A method for producing an infectious attenuated RSV particle from one or  
2     more isolated polynucleotide molecules encoding said RSV, comprising:
- 3             expressing in a cell or cell-free lysate an expression vector comprising an isolated  
4     polynucleotide comprising a recombinant RSV genome or antigenome which is modified  
5     by a partial or complete deletion of M2 ORF2 or one or more nucleotide changes that  
6     reduce or ablate expression of M2 ORF2, and RSV N, P, L and RNA polymerase  
7     elongation factor proteins.
- 1           74.     The method of claim 73, wherein the recombinant RSV genome or  
2     antigenome and the N, P, L and RNA polymerase elongation factor proteins are expressed  
3     by two or more different expression vectors.
- 1           75.     An isolated infectious recombinant respiratory syncytial virus (RSV)  
2     comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a  
3     nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase  
4     elongation factor, wherein the M2-2 ORF is transposed in the genome or antigenome to a  
5     more promoter-proximal or promoter-distal position compared to a native M2-2 gene  
6     order position to up-regulate or down-regulate, respectively, expression of the M2-2 ORF,  
7     or wherein the M2-2 ORF is incorporated in the genome or antigenome as a separate gene  
8     having a gene start and gene end gene end signal to alter expression of the M2-2 ORF.
- 1           76.     A method for producing one or more purified RSV protein(s) comprising  
2     the steps of:
- 3             infecting a host cell permissive of RSV infection with a recombinant RSV having  
4     a modification introduced into the genome or antigenome that comprises a M2-ORF 2  
5     deletion or knock out mutation under conditions suitable for RSV propagation;

6 isolating the recombinant RSV from the host cell; and  
7 purifying said one or more RSV protein(s) to yield a purified RSV protein sample.

1 77. The method for producing one or more purified RSV protein(s) according  
2 to claim 76, wherein the purified protein(s) comprises one or more viral antigen(s).

1 78. The method for producing one or more purified RSV protein(s) according  
2 to claim 77, wherein the purified protein(s) comprises one or more RSV F and/or G  
3 glycoprotein(s) or immunogenic domain(s) thereof.

1 79. The method for producing one or more purified RSV protein(s) according  
2 to claim 76, wherein the recombinant RSV expresses one or more viral protein(s) at a  
3 level that is approximately 2- to 3-fold greater than a level of expression of said one or  
4 more protein(s) by a wild-type or parental mutant RSV.

1 80. The method for producing one or more purified RSV protein(s) according  
2 to claim 76, wherein said one or more proteins is/are purified by chromatography using  
3 one or more immobilized antibody(ies) that specifically bind(s) to said one or more  
4 protein(s).

1 81. The method for producing one or more purified RSV protein(s) according  
2 to claim 76, wherein said recombinant RSV is further modified by a mutation that  
3 specifies a change to said one or more protein(s) that alters protein immunogenicity,  
4 solubility, and/or reactogenicity.

1 82. The method for producing one or more purified RSV protein(s) according  
2 to claim 76, wherein said purified RSV protein sample includes a purified RSV G protein.

1 83. The method for producing one or more purified RSV protein(s) according  
2 to claim 82, wherein the recombinant RSV is further modified by a mutation that  
3 comprises a deletion of an immunopathogenic domain located between amino acids 187  
4 and 200 of said RSV G protein.

1 84. The method for producing one or more purified RSV protein(s) according  
2 to claim 76, wherein said recombinant RSV is further modified by a mutation that further  
3 increases expression of said one or more RSV proteins.

1           85.     The method for producing one or more purified RSV protein(s) according  
2 to claim 84, wherein said mutation that further increases expression of said one or more  
3 RSV proteins includes one or more attenuating mutation(s) identified in a RSV 248/404  
4 mutant.

1           86.     The method for producing one or more purified RSV protein(s) according  
2 to claim 76, wherein said host cell is selected from HEp-2, FRhL-DBS2, MRC, or Vero  
3 cells.

1           87.     An isolated infectious recombinant respiratory syncytial virus (RSV)  
2 comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a  
3 nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase  
4 elongation factor, wherein the genome or antigenome incorporates an amino acid  
5 substitution at Asn43 of the RSV polymerase gene L.

1           88.     The isolated infectious recombinant RSV of claim 87, wherein Ile is  
2 substituted for Asn43.